Neural Grafting in the Mammalian CNS
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Solid Neural Grafts in Intracerebral Transplantation Cavities

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I. Introduction

In our laboratory the idea of transplanting central nervous system (CNS) tissue to the CNS stemmed from our previous experience with non-neuronal peripheral tissue transplanted to the CNS (Björklund and Stenevi, 1971; Svendgaard et al., 1975, 1976). In most of these experiments the iris was used as the transplant tissue and we studied the regenerative capacity of different CNS pathways by their ability to reinnervate this denervated target tissue. We learned from these studies that several aminergic and non-aminergic neuronal systems in the CNS had a true regenerative ability. Furthermore, they were able to form functional contacts with the smooth muscle in the reinnervated tissue (Björklund et al., 1975). Equally important was the finding that the transplanted iris was rapidly vascularized, particularly from the pial vessels but also from parenchymal vessels (Svendgaard et al., 1975). Blood vessels could be shown to enter the transplant during the first one to two days, and a capillary network of about half-normal density was re-established during the first five days. We also showed that the target tissue determined the properties of the newly formed blood vessels. Thus, a blood-brain-barrier developed in CNS transplants, whereas no such barrier could be found in peripheral transplants, irrespective of the origin of the blood vessels (Svendgaard et al., 1975).

Parallel to these studies Das and Altman (1971, 1972) and Olson and Seiger (1972) published their first observations on grafts of CNS tissue to the neonatal cerebellum and the anterior eye chamber, respectively. Inspired by their positive results we turned our attention to CNS transplants to the CNS. In this work our previous experience with iris transplants turned out to be extremely useful. In particular, we knew from the iris experiments that the blood vessels in the choroidal fissure are among the most efficient in revascularizing grafts to the CNS. Thus we chose to use this vascular surface as a 'culture bed' for the graft tissue. This was formed by making aspirative transplantation cavities in the cortex, exposing the choroidal fissure. It turned out that this created excellent conditions for graft survival and growth. We tried a variety of other techniques as well, but with limited success. In particular we found in these initial studies that in adult recipients, 'injected' transplants of solid CNS tissue resulted in large necrotic areas around the transplant with generally poor survival of the grafted tissue. By contrast, other groups (see Das, this volume) have shown that in the neonatal recipients CNS transplants can be injected into the depth of the brain parenchyma with excellent success.

II. 'Do-it-yourself' Brain Grafting

Before describing the techniques step-by-step, a few general points should be made. It is possible but impractical to do CNS transplants by oneself. Working in teams of two enables one person to prepare the graft while the other prepares the recipient animal, which makes the surgery run smoother and faster. This avoids any unnecessary handling of the graft, and risk factors, such as contamination and drying of the graft, are minimized. The person handling the recipient rat is able to concentrate fully on preparation of the cavity and in this way the time of surgery is kept to a minimum. Although sterile surgical conditions are not employed, clean operating conditions are desired and all instruments that handle the graft or the transplantation cavity are kept sterile and changed frequently during a transplantation session. No pre- or post-operative drug treatment is given under routine conditions.

III. Procedures

III.1. Preparation of the graft

The fetal donor brain is removed from the skull and transferred to a black glass cup filled with sterile saline. In this cup the desired piece or pieces are

dissected using Vannas' scissors. (For a detailed description of the fetal dissection and the selection of optimal fetal stages for different CNS regions, see Seiger, and Brundin et al., this volume.) The desired region should be dissected carefully in order to avoid contamination with surrounding mesodermal tissue. Remove as much of the pia as possible from the piece to be grafted. When the transplantation cavity is ready, which in an experienced team will be simultaneous to when the graft is prepared, the graft is picked up on a pointed scalpel blade or microspatula in a small drop of saline. The graft is then slid into the cavity with or without the help of watchmaker's forceps. This technique requires very little handling of the graft itself. We routinely use grafts in the range of 0.5–2 mm^3, but there do not seem to be any absolute upper or lower size limits.

III.2. Preparation of the transplantation cavity

For optimal control we place the anesthetized rat in a stereotaxic frame and all surgery is carried out under an operating microscope. One key to success in microsurgery is to be able to see well and thus to control every step of the operation. Next the skull is exposed through a longitudinal incision of the skin. With a dental drill the bone overlying the cortical area where the cavity is to be produced is removed. Always remove more bone, i.e. expose a larger area, than necessary, to provide an optimal view of the operating field. When drilling away the bone leave the innermost layer intact; this thin sheet of bone is then easily removed by forceps. The dura and cortex are then exposed and any bleeding is stopped with gelfoam (Spongostan, Ferrosan, Denmark). The dura is then opened with a pointed scalpel blade. With a bent suction glass pipette (Fig.1), the dural flaps are picked up and folded into the surrounding bone. The flaps will dry to the bone and not interfere with the rest of the surgery. Moist gelfoam can be used to prevent dehydration of the dural flaps. The cavity is created by suction. We prefer a glass pipette for optimal visibility. The pipette is connected to a suction apparatus and the suction force is regulated through the 'ventilation hole' on the pipette. When the ventilation hole is open minimal suction is obtained and only fluid is aspirated. In this fashion blood can be removed from the cavity and bleeding stopped without causing damage to the surrounding tissue. When the 'ventilation hole' is occluded with the index finger, maximal aspiration is obtained. In this way brain tissue can easily be removed in a very controlled fashion. The tip of the pipette should never be forced into the brain parenchyma but rather placed gently against the tissue to be removed. When the size of the pipette opening and the suction force of the suction apparatus is well balanced (determining this may require some practice) the surgeon is in total control of what is being aspirated down to the last 0.1 mm.

The size of the cavity is usually about 3×3 mm, but can be varied without jeopardizing the survival of the graft. Before the cavity is used for harboring a graft (or several grafts), check (1) that either the choroidal fissure or the vascularized surface of the thalamus-colliculi is exposed and constitutes the bottom of the cavity; and (2) that there is no ongoing bleeding in the cavity. Bleeding at any time during surgery is easily stopped with pieces of gelfoam soaked in sterile saline. In our own experiments we have worked with two types of cortical cavities, illustrated in Fig 1.

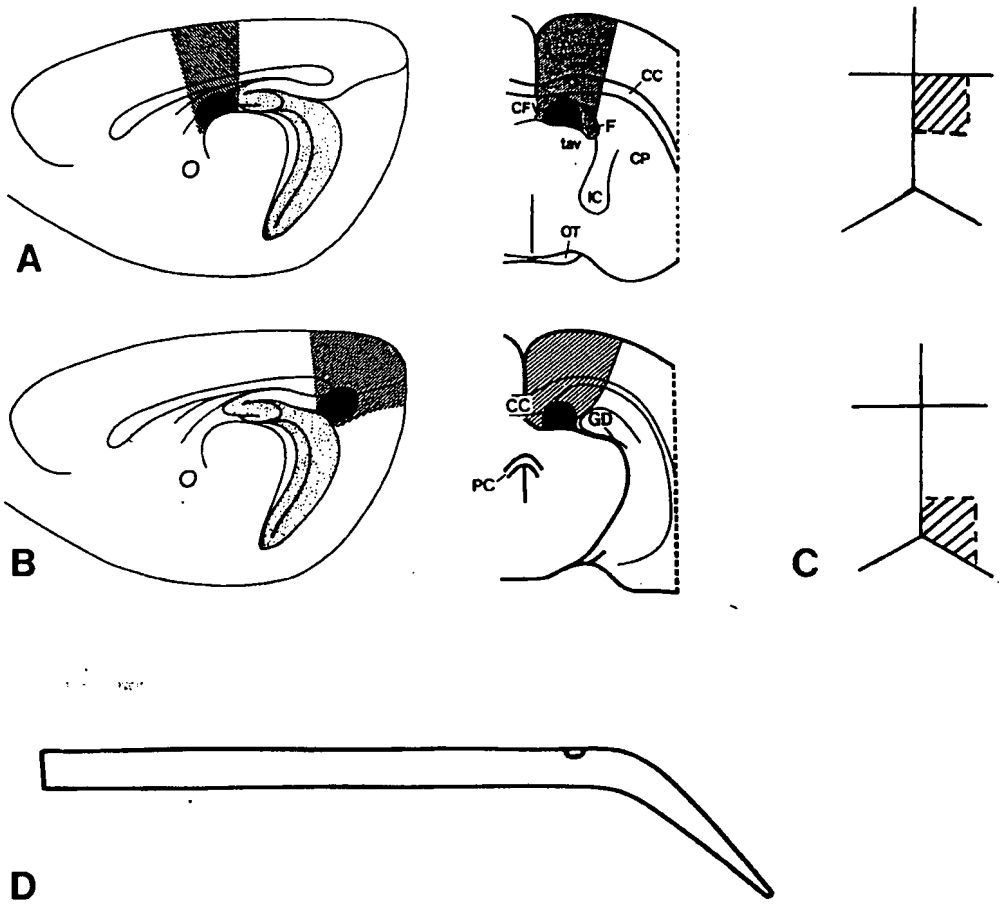

Figure 1. Extents of lesions (cross-hatching) and positions of the transplants (black) in the fornix-fimbria (A) and the occipital-retrosplenial cortex (B). The left drawings show the brain hemisphere in a medial view after the diencephalon brain stem has been removed. The right drawings show coronal sections through the transplantation site. Abbreviations: CC, corpus callosum; CFV, ventral fornix commissure; CP, nucleus caudatus-putamen; F, fimbria; GD, dentate gyrus; IC, internal capsule; OT, optic tract; PC, posterior commissure; tav, anteroventral thalamic nucleus. C shows schematic drawings of the rat skull seen from above. The marked areas denote the size and location of the opening in the bone and their relations to bregma and lambda. D is a schematic illustration of the design of the glass pipette, one-half actual size.

(a) *Caudal cortical site* on the pia overlying the superior colliculus. Open the bone according to the schematic illustration in Fig. 1C, thus exposing the desired cortical area. Start suction from behind removing the retrosplenial cortical tissue and exposing the choroidal fissure. The cavity extends medially to the midline, anterior to the presubiculum or dentate gyrus and laterally until the lateral ventricle is opened, i.e. approximately 3 mm lateral to the midline. This cavity transects entirely the perforant path input to the dorsal hippocampal formation.

(b) *Rostral cortical site* between septum and hippocampus, transecting the fornix and the hippocampal fimbria. Open the bone as shown in the schematic illustration in Fig. 1C. Start vertically through the cortical tissue. The white structure of the corpus callosum is an excellent landmark overlying the hippocampus. With the septal pole of the hippocampus exposed start transecting the fimbria from the medial side and go laterally until the septal part retracts forward, the sign of a complete transection. This cavity also transects all the dorsal routes of cholinergic, noradrenergic and serotoninergic afferents to the hippocampus (see Gage et al., 1983) as well as the major commissural connections running in the hippocampal commissure.

III.3. Transplantation to the cavity

If the cavity is properly prepared the surgeon should have an excellent view of the operating field. The graft, on the tip of a pointed scalpel blade or microspatula in a drop of saline (see above), is then easily transferred to the bottom of the cavity where it can be oriented by the surgeon. Surplus fluid is removed by gentle suction away from the graft when the proper position is attained. The graft is now covered with gelfoam, which will keep it in position and at the same time stop eventual bleeding. Surgery is completed when the skin is sutured. There is no need to suture the dura back in position since it will reform anyway on top of the gelfoam. The gelfoam is slowly absorbed in a period of about 6–12 weeks, although remnants may remain for longer times. The survival rate of a wide range of CNS regions has consistently been over 90% with this procedure.

III.4. The delayed transplantation technique

When the direct approach described above was tried in areas outside the choroidal fissure or the spinal cord the survival rate dropped significantly. In an effort to mimic the conditions in the choroidal fissure, we first attempted to construct cavities with vascular bridges using peripheral tissues such as iris. These vascular bridges extended from a pial vessel-rich area to the cavi-

ty removed from the choroidal fissure. Although this approach worked well (Stenevi et al., 1976), later experiments showed that similar conditions are obtained by simply delaying the transplantation to a cavity prepared several weeks earlier (Stenevi et al., 1980). The cavity is performed as described above and filled with gelfoam. In a second operation the cavity is re-opened and the gelfoam gently removed by suction. The newly formed blood vessels on the walls of the cavity (partly formed as a result of down-growth of pia into the cavity) will now serve a similar function as the blood vessels in the choroidal fissure. After bleeding is eventually stopped and the graft is placed in position, the rest of the cavity is filled with gelfoam. We have applied this technique extensively in experiments with grafts of embryonic substantia nigra in cavities prepared in the cortex overlying the caudate-putamen. This has yielded excellent results with an overall graft survival rate of 90% or better (Björklund and Stenevi, 1979; Björklund et al., 1980; Dunnett et al., 1981a-c; Freund et al., 1984). Properly performed this delayed technique yields excellent results, although every additional surgery will increase the risk of infections, bleeding, respiratory failure and other adverse reactions to surgery. In our own experience the optimal time interval is between 2 and 12 weeks. After 6 weeks the remaining gelfoam is very easy to remove without causing any additional damage to the surrounding tissue or additional bleeding.

III.5. Composite grafts

One advantage of the present technique is that more than one transplant can be placed with control in the same cavity. The number of combinations are seemingly endless, thus 'miniature brains' (Möllgaard et al., 1978) or neuronal model systems can be constructed in this way. For example, selected neuronal populations and their target tissues can be placed in close contact, or separated from each other by other types of tissues, or they can be kept apart by thin strips of gelfoam. In our own studies (unpublished) we have combined pieces of fetal brain stem (locus coeruleus or raphe region) with pieces of fetal hippocampus or cortex. The two parts have been seen to fuse and the noradrenergic and serotoninergic neurons in the brain stem parts have been seen to reinnervate the cortical tissue. We have also combined fetal hypothalamus with fetal pituitary with good results (Paden et al., 1980, and unpublished results).

In other experiments (Aguayo et al., 1984) embryonic nigral grafts (placed in the caudal cavity, see above) have been combined with segments of peripheral nerve, placed so that they connect the nigral graft with the host caudate-putamen, and run over the parietal cortex. Axons growing from the grafted dopamine neurons have in this way been possible to link extracerebrally all the way to the striatum along the nerve.

IV. Comments

IV.1. Age of host

Although our experience so far with the current technique has chiefly been with young adult rats, we have obtained excellent results also in neonatal, young postnatal and old rats. This is also evident from the work of Lund and collaborators (see this volume) with grafts of CNS tissue to the superior colliculus in neonate rats, where they also have taken advantage of the excellent features of the choroidal fissure for graft survival. In their work, however, they have inserted the graft tissue directly into the choroidal fissure, without removing any overlying cortical tissue.

IV.2. Age of donor

Although most regions of the neuroaxis will survive grafting after a wide range of fetal (and in some cases also neonatal) donor ages (cf. Olson et al., this volume), the final size and the intrinsic organizational features of the grafted tissue will vary greatly depending on the developmental stage of the region. In general, young donor tissues grow to become larger than late gestational tissues. In the study of Kromer et al., (1983), early gestational hippocampal grafts grew as much as 3800% while late gestational cerebellar tissue decreased in size. The ability of the graft to develop its characteristic intrinsic organization and three-dimensional architecture is also dependent on the donor age. For further discussion of these points the reader is referred to the papers of, for example, Wells and McAllister (1982), Alvarado-Mallart and Sotelo (1982) and Kromer et al. (1983).

IV.3. Grafting to the spinal cord

The cavity grafting technique is also applicable to the spinal cord. Nornes et al. (1983) have shown that the pia surrounding the spinal cord is a good 'culture bed' for CNS tissue grafts. In their experiments a suction cavity (1–3 mm^3 in size) was made subpially by removing $\frac{1}{3}$–$\frac{2}{3}$ of a cord segment, and the graft was inserted in contact with the spared pial covering. The survival and growth of fetal brain stem grafts were similar to those seen with similar transplants in cortical cavities (see Nornes et al., 1983, for further details).

V. Pros and Cons of the Cavity Grafting Technique

The limitations of the cavity grafting technique are primarily related to the use of a transplantation cavity. By necessity this involves removal of tissue,

e.g. the cortex, and the method is thus more traumatic to the host brain than, for example, the suspension grafting method described elsewhere (see Brundin et al., this volume). Furthermore, the cavity grafting method is limited to areas close to the external surface of the CNS.

The main advantages of the technique can be summarized under the following points.

(1) Since the grafting is done under visual guidance the placement and orientation of one or several individual grafts can be well controlled.

(2) The graft is easily accessible from outside, also after long survival times, which allows, for example, tracer injections or electrophysiological recordings within selected portions of the graft to be made under direct visual control. Moreover, the graft is in most cases well delineated so that it can be lesioned or removed by suction for the purpose of, for example, functional studies or axonal tracing with anterograde degeneration methods.

(3) The cavity method allows the researcher to regulate, at least to some extent, the degree of anatomical interaction between the graft and the host brain. Thus, the graft can be made to fuse with the adjoining host brain tissue (which facilitates the re-establishment of afferent and efferent axonal connections), or it can be isolated from the host with, for example, sheets of gelfoam. In the latter case the technique comes close to an intracerebral culture system with interesting potentials for developmental and functional studies (see, e.g., Möllgaard et al., 1978; Paden et al., 1980; Alvarado-Mallart and Sotelo, 1982; Kromer et al., 1979, 1983).

(4) Correctly positioned, the cavity can serve the combined purpose of harboring the graft and providing a desired denervation of the target tissue (such as is the case with the rostral and caudal cortical sites in Fig.1).

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